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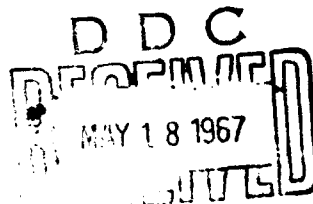
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TECHNICAL MANUSCRIPT 384

CORROSION AND REDUCTION OF IRON  
BY A SOLUBLE DEHYDROGENASE PRODUCED  
BY DESULFOVIBRIO DESULFURICANS

Warren P. Iverson

MAY 1967



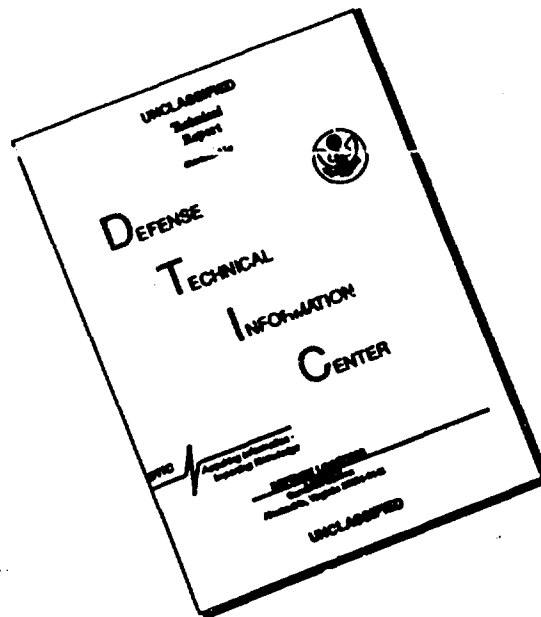
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CORROSION AND REDUCTION OF IRON BY A SOLUBLE DEHYDROGENASE  
PRODUCED BY DESULFOVIBRIO DESULFURICANS

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Special Operations Division  
COMMODITY DEVELOPMENT AND ENGINEERING LABORATORY

Project 1C522301A061

May 1967

CORROSION AND REDUCTION OF IRON BY A SOLUBLE DEHYDROGENASE  
PRODUCED BY DESULFOVIBRIO DESULFURICANS

ABSTRACT

Desulfovibrio desulfuricans liberates a soluble hydrogenase that is capable of reducing  $\text{Fe}^{++}$  to elemental iron in a hydrogen atmosphere. The classical cathodic depolarization theory of bacterial corrosion is modified to account for this finding. The corrosion rate by this mechanism appears to be very small.

The classical theory to account for anaerobic bacterial corrosion, postulated by von Wolzogen Kühr and van der Vlugt,<sup>1</sup> states that certain organisms, primarily those of the genus Desulfovibrio, remove hydrogen (electrons) that accumulates on the surface of iron (cathodic depolarization) by means of a hydrogenase and reduce  $\text{SO}_4^{--}$  to  $\text{S}^{--}$ . As a result of electron removal, iron dissolves as  $\text{Fe}^{++}$  ions at the anode.

It was recently demonstrated that a strain of D. desulfuricans would cathodically depolarize mild steel in a nitrogen atmosphere if the electron acceptor was the redox dye benzyl viologen (BV).<sup>2</sup> A 1010 steel coupon was placed on a 2% agar surface containing BV and tris buffer [tris(hydroxymethyl)aminomethane] at  $\text{pH } 7.0 \pm 0.1$  so that half of the coupon (cathode) rested on a mass of cells of D. desulfuricans, Mid-Continent strain A. The BV was reduced by the cells, and the iron dissolved as  $\text{Fe}^{++}$  ions at the half of the coupon (anode) not in contact with the cells. In summary, the reaction at the anode is  $\text{Fe} \rightarrow \text{Fe}^{++} + 2\text{e}^-$ ; at the cathode  $2\text{e}^- + 2\text{H}^+ \rightarrow 2\text{H} \rightarrow \text{H}_2$ ;  $\text{H}_2 + 2 \text{BV} \rightarrow 2 \text{BV}_e + 2\text{H}^+$ .

If a coupon was placed on 2% yeast extract (YE) agar,  $\text{pH } 7.0 \pm 0.1$ , plus 0.01% BV with cells under one-half of the coupon, the results appeared to be the same. A depolarization current of 2  $\mu\text{amp}$  per  $\text{cm}^2$  was obtained by the technique previously reported.<sup>3</sup> When the experiment was repeated without BV in the YE agar, the agar blackened under the half of the coupon in contact with the cells. The black area became blue-green when developed with aqueous potassium ferricyanide solution (10% w/v), indicating the presence of  $\text{Fe}^{++}$  ions. No  $\text{Fe}^{++}$  ions were found in the agar under the half of the coupon not in contact with the cells (Fig. 1). The black area, which extended several millimeters into the agar, appeared to consist of particles less than 1 micron in diameter. Black particles were also found in the cells. After the cells were treated with the potassium ferricyanide solution, blue-green areas were found in the cells, indicating local concentrations of  $\text{Fe}^{++}$ .

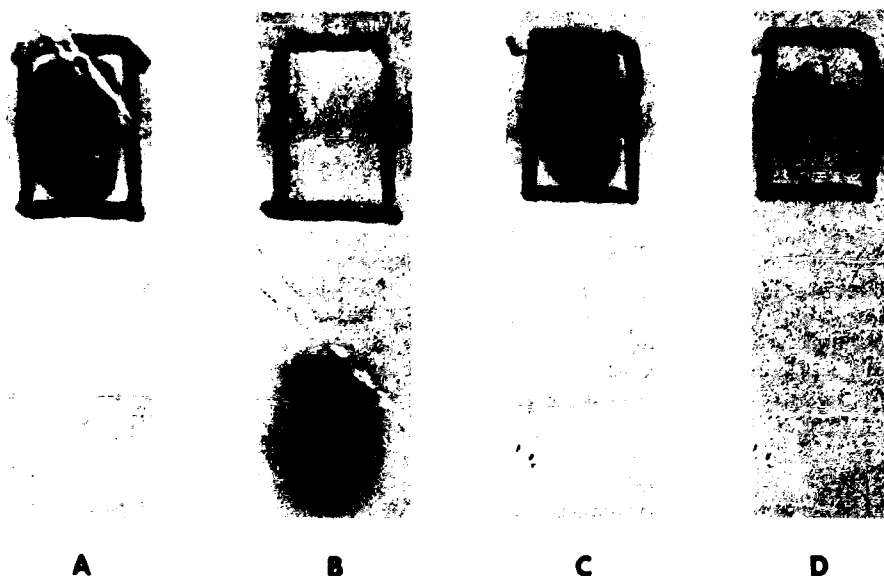


Figure 1. Areas in Agar under Coupon Indicating Location of Iron and Reduced BV. A. Yeast extract (YE) agar plus BV surface immediately after removal of steel coupon. Dark area (cathode) due to reduction of BV by *Desulfovibrio* cells. B. Same plate 15 minutes after addition of aqueous potassium ferricyanide (10% w/v) indicating a concentration of  $\text{Fe}^{++}$  ions at the anode (no cells). C. YE agar minus BV immediately after removal of steel coupon, showing dark area under cells. D. Same plate 15 minutes after addition of aqueous potassium ferricyanide showing concentration of  $\text{Fe}^{++}$  ions.

Some material other than BV, apparently iron, was being reduced. A similar but less intense blackening of the cells and agar was also observed under the half of the coupon in contact with the cells when 0.5% NaCl was present in tris buffer agar without redox dye. If 0.01% methylene blue (MB) was added to the NaCl - tris buffer agar, blackening again occurred under the half of the coupon in contact with the cells, but the reduction of MB was considerably less than that in the absence of NaCl. This seemed to indicate a competition between MB and the other species, presumably  $\text{Fe}^{++}$  ions as electron acceptors.

When 0.2%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  was substituted for BV, as an electron acceptor, in YE agar the results appeared to be the same as with YE agar alone. The cells and agar under the coupon again became black. The substitution of 0.01%  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  for BV in tris buffer agar without NaCl produced no cathodic depolarization effect, that is, no apparent increase in iron concentration at the anode compared with a control system without cells. No sustained cathodic depolarization current was obtained in either of these systems, and the polarity of the system was unstable in that the polarity continually reversed. Apparently the cells were reducing  $\text{Fe}^{++}$  ions and a short circuit was occurring in the system in that both anodic and cathodic reactions were occurring at the area of the steel coupon that was in contact with the cells.

When cells of the same strain grown on a trypticase soy broth plus agar surface<sup>3</sup> were placed on the surface of petri plates of washed agar (Noble, Difco) containing tris buffer at  $\text{pH } 7.0 \pm 0.1$ , 0.5% NaCl and 1.75 mg  $\text{FeCl}_2$  per ml in a hydrogen atmosphere, blackening of the cells was noted within 12 hours and often in less than 2 hours. Black particles were again found within the cells. No blackening of the cells was noted in a nitrogen atmosphere. Sterile 2% YE broth of  $\text{pH } 7.0 \pm 0.1$  containing a steel coupon, when inoculated with a pure culture and incubated in a hydrogen atmosphere, often became black within 24 hours, depending on the effectiveness of oxygen removal. A clump of cells on the surface of the steel coupon often became black within an hour or less. In a nitrogen atmosphere, blackening was often delayed by a day or two. The same results were observed with three other cultures of Desulfovibrio, one of which was isolated from a tubercle in a water pipe, and a Clostridium species isolated from tap water. The surface of the steel coupon generally remained as bright as when it was placed in the YE broth except where it came in direct contact with the black sediment at the bottom of the tube.

An attempt was made to measure this corrosion in tubes of inoculated 2% YE broth (10 ml per tube) using a corrosion probe\* of 1010 steel with a Corrosometer.\*\* The operation of the Corrosometer is based on the fact that the electrical conductivity of most metals is very great compared

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\* Model 8001, S4, Magna Corp., Santa Fe Springs, Calif.

\*\* Model CK, Crest Instrument Co., Santa Fe Springs, Calif.



with the negligible conductivity of nonmetals. As the corrosion process converts metal into nonmetal, the electrical resistance of the exposed metal coupon in the probe increases. The resistance of the exposed specimen is compared with that of a second protected specimen of the same alloy using the sensitive bridge circuit of the Corrosometer.<sup>4,5</sup> After several weeks' incubation at  $26 \pm 1^\circ\text{C}$ , no detectable difference in resistance in the probe specimen could be noted between three probes in inoculated YE broth ( $0.14\text{ mg SO}_4^{--}$  as the barium salt per ml) and three probes in sterile YE broth. One of the inoculated tubes with probe and a sterile control tube are shown in Figure 2. Because the corrosion rate was less than  $2.6\text{ mg per dm}^2\text{ per day}$  (limit of sensitivity of meter for a 3-day interval), it may have been equal to or less than the corrosion rate established for the cathodic depolarization of 1010 steel using BV as the electron acceptor ( $2.5\text{ mg per dm}^2\text{ per day}$ ). Corrosion of probes could not be detected in 4 weeks in inoculated YE plus  $0.2\%$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and YE plus  $4\text{ ml per liter}$  sodium lactate and  $0.2\%$   $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .

It was suspected from observations made during the isolation of Desulfovibrio from tubercles in a water main that the blackening of the YE broth might be produced by a cell-free enzyme liberated in the medium. Using the Thunberg technique, Seitz filtrates (cell-free) of 3-day-old YE broth cultures of the four strains of Desulfovibrio and the Clostridium sp. containing a steel coupon in a hydrogen atmosphere reduced  $\text{FeCl}_2$  ( $E_0 = -440\text{ mv}$ ) (blackening due to colloidal iron), methyl viologen ( $E_0 = -411\text{ mv}$ ), and benzyl viologen ( $E_0 = -315\text{ mv}$ ). No reduction was observed in a nitrogen atmosphere. A sterile 1010 steel coupon in the Seitz filtrate of these strains in a hydrogen atmosphere produced blackening of the filtrate, usually within 24 hours, if most of the oxygen was removed. In the same system under a nitrogen atmosphere, a flocculent black precipitate first formed on the surface of the metal. Complete blackening of the filtrate was delayed for about 24 to 48 hours compared with the time of blackening in a hydrogen atmosphere. Apparently the small amount of hydrogen on the steel surface initially limited the reaction. A sterile Seitz filtrate of a 3-day-old blackened YE broth culture of the Mid-Continent strain in contact with 1010 steel coupons under  $\text{H}_2$  was again allowed to react with sterile 1010 steel coupons. After 3 days under a  $\text{H}_2$  atmosphere a black precipitate was obtained by centrifugation under hydrogen. X-ray diffraction analysis\* of this material was negative for elemental iron or any compound of iron, indicating that the colloidal iron was probably in an amorphous state. Similar results were noted when bismuth and antimony were reduced by bacteria.<sup>6</sup> A few magnetically attracted particles were produced, however, from cells grown in the presence of  $\text{Fe}^{++}$  ions or a steel coupon that had been rapidly frozen and thawed three or four times.

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\* By Dr. J. Kruger, National Bureau of Standards.

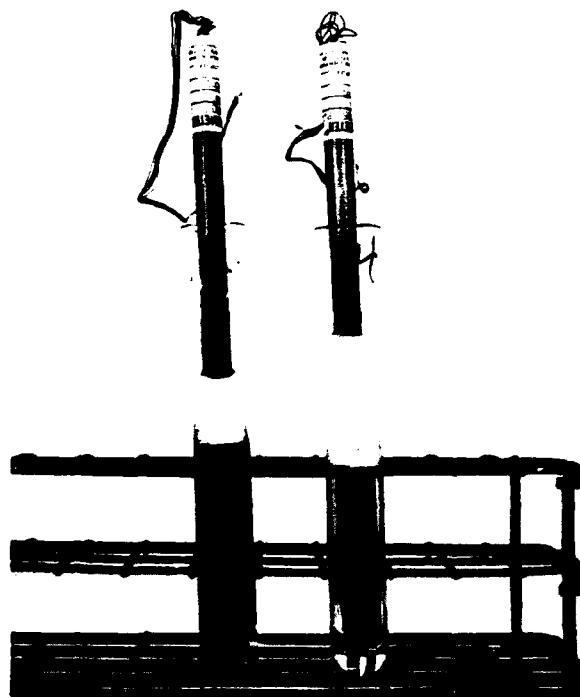


Figure 2. Two Corrosion Probes (1010 Steel) in 2% Yeast Extract ( $\text{pH } 7.0 \pm 1$ ) Sealed with Vaspar (Equal Portions of Vaseline and Paraffin). Tube at left inoculated with culture of *D. desulfuricans* (Mid-Continent strain A). Tube at right uninoculated control. After two days' incubation at  $26 \pm 1$  C.

It has appeared that Desulfovibrio produced a soluble extra cellular hydrogenase that is capable of reducing  $\text{Fe}^{++}$  ions to Fe in the presence of hydrogen. The corrosion of iron by a modification of the classical cathodic depolarization theory of von Wolzogen Kühr and van der Vlugt may therefore be summarized by the following reactions:



Although the rate of corrosion by this mechanism appears to be very low, on initial observation it seems high as evidenced by the intense darkening of the surrounding medium because of colloidal iron. Because a Seitz filtrate of organisms in YE broth under a seal of vaspar in the absence of iron, where growth is normally very poor, also reduced iron, growth of the organisms in the presence of iron does not appear to be necessary for hydrogenase production.

The ability of several microorganisms to reduce metal ions has been reported previously.<sup>7,8</sup> One might postulate that the colloidal metal in the cell is oxidized, thus providing a source of energy for the cell. Because Desulfovibrio has been found in association with tubercles in iron water pipes, further establishment of the relationship between the two would be significant. A comparison of the properties of the soluble dehydrogenase with those of the cell-extractable dehydrogenase reported for Desulfovibrio<sup>9</sup> appears to be of interest. In view of the above results, it seems that the practice of placing a nail in medium used for the cultivation of anaerobes (especially Clostridium species) to indicate  $\text{H}_2\text{S}$  production (blackening of the medium being assumed to be due to  $\text{FeS}$  production) should be reexamined. The organisms may be producing colloidal iron rather than  $\text{H}_2\text{S}$  and  $\text{FeS}$ . As it is well established that sulfides are produced in the vicinity of iron pipes by sulfate reduction, an examination of the environmental factors and reduction products will be necessary to establish what selectivity the organisms have for the reduction of  $\text{Fe}^{++}$  and  $\text{SO}_4^{--}$ .

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